Nerve Growth Factor Is Increased in Psoriatic Skin

To the Editor:

Several evidences indicate that neurogenic factors could take part in the pathogenesis of psoriasis. Recently, marked changes in the cutaneous levels of the neuropeptides (NP) substance P (SP) and vasoactive intestinal peptide have been radioimmunologically demonstrated in psoriatic plaques as compared to healthy skin [1]. It is known that similar reactive alterations in the NP content can also be observed in the sensory ganglia after peripheral nerve injury [2] or after peripheral inflammation in rats [3]. The neurotrophin nerve growth factor (NGF) is considered to be a primary candidate as a regulatory molecule in these peptidergic responses. Indeed, 1) NGF is increased in the nerves supplying inflamed skin, 2) injection of NGF in the skin reproduces the same neuronal peptidergic modifications observed during experimental inflammation in rats, 3) pre-treatment with anti-NGF serum prevents the NP changes at a neuronal level [4]. Furthermore, in human skin, NGF levels dramatically increase in blister exudates [5]. It is therefore tempting to speculate that NGF could play an important role in spontaneous inflammatory dermatoses such as psoriasis, by modulating NP alterations.

Against this background, we have measured NGF levels in biopsies from psoriatic skin. Specimens were taken from both lesional (n = 19) and corresponding non-lesional (n = 20) skin of the dorsum in patients with plaque psoriasis. Biopsies were also taken from the skin of healthy subjects (n = 11) as controls. NGF was measured in skin homogenates using a two-site enzyme-linked immunosorbent method, specific for human NGF [6]. A significantly increased amount of NGF was detected in lesional psoriatic skin (77.1 ± 6.7 pg/g tissue), as compared to both non-lesional (59.6 ± 4.7 pg/g tissue) and control skin (47 ± 4.6 pg/g tissue) **(Fig 1)**.

NGF is synthesized and released in the skin by proliferating keratinocytes [7]. Therefore, hyperproliferating keratinocytes are likely to be responsible for the increased NGF levels in psoriatic skin. NGF regulates the synthesis and expression of NP in mature sensory neurons [8]. NGF could be released by keratinocytes, uptaken and retrogradely transported in cutaneous nerve fibers [9], which bear NGF receptor [10], thus inducing changes in NP synthesis in the corresponding primary sensory neurons.

We have recently reported that NGF stimulates keratinocyte proliferation through binding to high-affinity receptor [7] and an autocrine loop for NGF in keratinocytes has been proposed [11]. In addition, NGF exerts a number of effects on immune-inflammatory cells [12,13]. In conclusion, NGF should be regarded as a cytokine, potentially intervening in different pathomechanisms leading to the generation and maintenance of the psoriatic lesion.

Accepted for publication September 15, 1995.

Fabrizio Fantini
Division of Dermatology
Ospedale Civile di Venezia
Venezia, Italy

Cristina Magnoni
Department of Dermatology
University of Modena
Modena, Italy

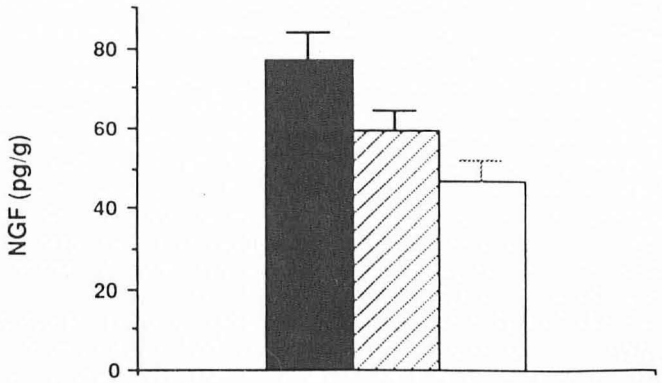

Figure 1. NGF levels in psoriatic skin. NGF was measured by a two-site ELISA method in psoriatic lesional (solid bar) and non-lesional skin (hatched bar), as well as in healthy skin (open bar). Values are given in pg/g tissue, and results represent means ± SEM from two experiments in triplicate. Lesional vs non-lesional skin $p < 0.05$; lesional vs healthy skin $p < 0.02$.

Luisa Bracci-Laudiero
Institute of Neurobiology, CNR
Rome, Italy

Carlo Pincelli
Department of Dermatology
University of Modena
Modena, Italy

REFERENCES

1. Pincelli C, Fantini F, Romualdi P, Sevignani C, Lesa G, Benassi L, Giannetti A: Substance P is diminished and vasoactive intestinal peptide is augmented in psoriatic lesions and these peptides exert disparate effects on the proliferation of cultured human keratinocytes. *J Invest Dermatol* 98:421–427, 1992
2. Villar M, Cortes R, Theodorsson E, Wiesenfeld-Hallin Z, Schalling M, Fahrenkrug J, Emson P, Hökfelt T: Neuropeptide expression in rat dorsal root ganglion cells and spinal cord after peripheral nerve injury with special reference to galanin. *Neuroscience* 33:587–604, 1989
3. Lembeck F, Donnerer J, Colpaert FC: Increase of SP in primary afferent nerves during chronic pain. *Neuropeptides* 1;175–180, 1981
4. Donnerer J, Schuligoi R, Stein C: Increased content and transport of substance P and calcitonin gene-related peptide in sensory nerves innervating inflamed tissue: evidence for a regulatory function of nerve growth factor *in vivo*. *Neuroscience* 49:693–698, 1992
5. Weskamp G, Otten U: An enzyme-linked immunoassay for nerve growth factor (NGF): a tool for studying regulatory mechanisms involved in NGF production in brain and in peripheral tissues. J Neurochem 48:1779–1786, 1987
6. Bracci-Laudiero L, Aloe L, Levi-Montalcini R, Buttinelli C, Schilter D, Gillessen S, Otten U: Multiple sclerosis patients express increased levels of beta-nerve growth factor in cerebrospinal fluid. *Neurosci Lett* 147:9–12, 1992
7. Pincelli C, Sevignani C, Manfredini R, Grande A, Fantini F, Bracci-Laudiero L, Aloe L, Ferrari S, Cossarizza A, Giannetti A: Expression and function of nerve growth factor and nerve growth factor receptor on cultured keratinocytes. *J Invest Dermatol* 103:13–18, 1994
8. Lindsay R, Harmar A: Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. *Nature* 337:362–364, 1989

0022-202X/95/$09.50 • SSDI0022-202X(95)00402-5 •

9. Raivich G, Hellweg R, Kreutzberg G: NGF receptor-mediated reduction in axonal NGF uptake and retrograde transport following sciatic nerve injury and during regeneration. *Neuron* 7:151–164, 1991
10. Fantini F, Johansson O: Expression of growth-associated protein 43 and nerve growth factor receptor in human skin: a comparative immunohistochemical investigation. *J Invest Dermatol* 99:734–742, 1992
11. Di Marco E, Mathor M, Bondanza S, Cutuli N, Marchisio PC, Cancedda R, De Luca M: Nerve growth factor binds to normal human keratinocytes through high and low affinity receptors and stimulates their growth by a novel autocrine loop. *J Biol Chem* 268:22838–22846, 1993
12. Pearce FL, Thompson HL: Some characteristics of histamine secretion from rat peritoneal mast cells stimulated with nerve growth factor. *J Physiol* 372:379–393, 1986
13. Otten U, Ehrhard P, Peck R: Nerve growth factor induces growth and differentiation of human B lymphocytes. *Proc Natl Acad Sci USA* 86:10059–10063, 1989

Th2 Cytokine Profile in Cutaneous T-Cell Lymphoma

To the Editor:

We read with great interest the article by Saed *et al* entitled "Mycosis fungoides exhibits a Th1-type cell-mediated cytokine profile whereas Sezary syndrome expresses a Th2-type profile" in the July 1994 issue of the Journal of Investigative Dermatology [1]. Using reverse-transcriptase–polymerase chain reaction (RT-PCR) amplification, the authors demonstrated the presence of mRNA for IL-2 and interferon-gamma (IFN-γ) but no evidence of mRNA for interleukin 4 (IL-4), IL-5, or IL-10 in the epidermis of seven patients with plaque-type mycosis fungoides (MF). This was in contrast to the epidermis (n = 3) and the blood (n = 7) of Sezary syndrome (SS) patients that demonstrated message for IL-4, IL-5, and IL-10, but no evidence of IL-2 or IFN-γ. The findings in the MF skin is in keeping with a Th1 cytokine pattern and the findings in the Sezary syndrome skin and blood are in keeping with a Th2 cytokine pattern. As the title of the article implies, the author's interpretation of these findings is that the malignant T cells of mycosis fungoides elaborate a different set of cytokines than those of Sezary syndrome. Our group has recently published data on similar studies in cutaneous lesions taken from different stages of cutaneous T-cell lymphoma (CTCL) [2]. Our data is in partial agreement with Saed *et al*; however, because of differences in our findings in normal controls, our interpretation and conclusion is that the malignant T cells in cutaneous T-cell lymphoma, both in mycosis fungoides and Sezary syndrome, are of a Th2 subtype based on the expression of the Th2 cytokines.

It must be first pointed out that RT-PCR amplification studies such as these provide a window into the miciro-environment of the skin but cannot determine the precise source of a particular amplification signal, as do *in situ* hybridization studies. To draw any conclusions concerning thee source of amplification signals from PCR experiments, normal controls are critical. We believe subtle differences in our techniques have led to different results in normal skin controls that have led to differences in interpretation of our respective data.

Unlike Saed *et al*, we found amplification signals for IL-2 and IFN-γ in 100% of the 12 normal skin controls we analyzed. Saed *et al* reports that they have detected IFN-γ mRNA in four of 11 normal skins, but no IL-2. We believe that our technique is more sensitive than that of Saed *et al*; this may be related to primer selection. In addition, we analyzed whole punch biopsies and did not separate epidermis and dermis by heating. Potentially, heating of the samples may have affected more labile transcripts. Because there are no endogenous skin cells that have been documented to secrete IL-2 or IFN-γ, including Langerhans cells [3], we interpreted our data to indicate that the source of the IL-2 and IFN-γ amplification products were from normal lymphocytes that traffic through the skin in an activated state [4]. Furthermore, we reasoned that our assay's detection threshold was sensitive enough to consistently detect these cells.

If IL-2 and IFN-γ, cytokines that counteract the expression and effects of Th2 cytokines, are present in detectable quantities in normal skin, then they may have the potential to down regulate IL-4 and IL-5 secretion from infiltrating Th2 cells. Furthermore, Th1 cytokines inhibit Th2 cytokine mRNA expression. The concept of immunoregulation of the malignant cells in early CTCL fits the natural history and indolent nature of the early stages of the disease. Studies have shown IFN-γ–secreting cytotoxic T cells to be present in the infiltrate of MF lesions [5] and these may influence the cytokine expression of malignant CD4 cells.

It is noteworthy that our studies provide clear evidence of Th2 cytokines in cutaneous MF lesions, particularly tumor stage disease in which the disease is aggressive and poorly regulated. In six of six biopies from cutaneous tumors from MF patients, we detected Th2 cytokine mRNA. Moreover, we also detected Th2 cytokine mRNA in three of six samples from patients with early patch- or plaque-stage disease. Thus, we interpret Th1 signals in early MF lesions to be a result of either normal trafficking lymphocytes or a tumor-infiltrating host response with a predominance of Th1 cytokines that may supress cytokine gene transcription of MF tumor cells.

The most cohesive, biologically relevant model to explain the Th1 findings in early MF is that CTCL is a malignancy of a Th2 CD4 T-cell that is down regulated in early disease by Th1 cells. This would explain why RT-PCR demonstrates a Th1 profile in early skin disease. This model excludes the unproved speculation that a human Th1 cell is capable of differentiating into a Th2 cell as suggested by Saed *et al*. We believe that the findings of the Th1 profile in both papers are compatible with a Th2 model of CTCL and that the attempt to use a dichotomous paradigm of cytokine secretion of mycosis fungoides and Sezary syndrome is misleading and inaccurate. *In situ* hybridization studies will hopefully resolve this issue with certainty.

S.R. Lessin
Philadelphia Veterans Affairs Medical Center &
Department of Dermatology
University of Pennsylvania

B.R. Vowels and A.H. Rook
Department of Dermatology
University of Pennsylvania
Philadelphia, Pennsylvania

REFERENCES

1. Saed G, Fivenson DF, Naidu Y, Nickoloff BJ: Mycosis fungoides exhibits a Th 1-type cell-mediated cytokine profile whereas Sezary syndrome expresses a Th 2-type profile. *J Invest Dermatol* 103:29–33, 1994
2. Vowels BR, Lessin SR, Cassin M, Jaworsky C, Benoit B, Wolfe JT, Rook AH: Th2 cytokine expression in skin of cutaneous T-cell lymphoma. *J Invest Dermatol* 103:669–673, 1994
3. Matsui H, Cruz PD, Bergstresser PR, Takashima A: Langerhans cells are the major source of mRNA for IL-1 beta and MIP-1 alpha among unstimulated mouse epidermal cells. *J Invest Dermatol* 99:537–541, 1992
4. Bos JD, Zonneveld I, Das PK, Kreig SR, van der Loos CM, Kapsenberg MT: The skin immune system (SIS): distribution and immunophenotype of lymphocyte populations in normal human skin. *J Invest Dermatol* 88:569–573, 1987
5. Wood GS, Edinger A, Hoppe RT, Warnke RA: Mycosis fungoides skin lesions contain CD8+ tumor-infiltrating lymphocytes expressing an activated, MHC-restricted cytotoxic T lymphocyte phenotype. *J Cutan Pathol* 21:151–156, 1994